SYNTHESIS AND EVALUATION OF NOVEL POLYMERIC PRODRUGS FOR THE TREATMENT OF COLON CANCER

1. Introduction:

1.1 Incidence, Prevalence and Survival

Cancer is the world's incurable and widely diagnosed disease in human beings. According to GLOBOCAN 2012 (new version of "The International Agency for Research on Cancer" online database) an estimated 14.1 million new cases of cancer diagnosed worldwide when compared with 2008, which would be estimated to rise up to 19.3 million new cancer cases per year by 2025. Cancer mainly affects the elderly. The most recent estimates of worldwide cancer burden indicate that about 45% of cancers occurred in people aged 65 years or over in 2002 **(Ferlay *et al.*, 2013; Masood, 2016).**

Approximately 4.6% of men (1 in 22) and 4.2% of women (1 in 24) will be diagnosed with colorectal cancer in their lifetime. Lifetime risk is similar in men and women despite higher incidence rates in men because women have longer life expectancy **(Siegel *et al.*, 2016).**

Colorectal cancer can begin in either the colon or the rectum. Cancer that begins in the colon is called colon cancer. Cancer that begins in the rectum is called rectal cancer. Cancer develops much less often in the small intestine than in the colon or rectum (colorectum). Colorectal cancer usually develops slowly, over a period of 10 to 20 years **(Winawer and Zauber, 2002)**. Most colon and rectal cancers are a type of tumour called adenocarcinoma, which is cancer of the cells that line the inside tissue of the colon and rectum. The most common kind of polyp is called an adenomatous polyp or adenoma. Adenomas arise from glandular cells, which produce mucus tolubricate the colorectum. An estimated one-third to one-half of all individuals will eventually develop one or more adenomas **(Bond, 2000; Schatzkin *et al.*, 1994).** Although all adenomas have the capacity to become cancerous, fewer than 10% are estimated to progress to invasive cancer **(Levine and Ahnen, 2006; Risio, 2010).** The likelihood that an adenoma will evolve into cancer increases as it becomes larger **(Pickhardt *et al.*, 2013).** Cancer that develops in glandular cells is called adenocarcinoma. Most colorectal cancers (approximately 96%) are adenocarcinomas **(Stewart *et al.*, 2006)**.

The physiological anatomy of colon cancer is shown in Figure 1.

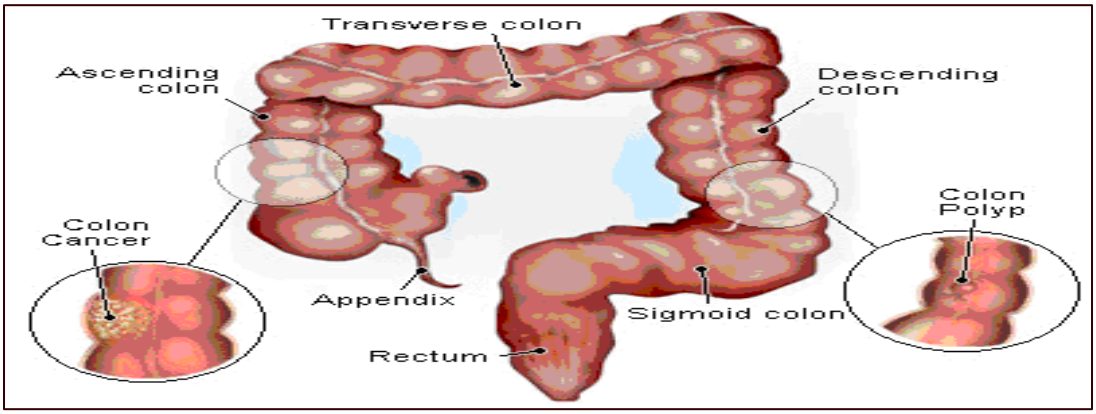

Figure 1: Physiological anatomy and schematic representation of colon cancer.

1.2 Stages of Colon Cancer

The two most common staging systems are: The TNM system, which is typically used in clinical settings, and The Surveillance, Epidemiology, and End Results (SEER) summary staging system, which is used for descriptive and statistical analysis of tumour registry data. Colorectal cancer stages using the SEER summary staging system **(Alteri *et al.*, 2016)** are as follows:

***In situ*:** Cancers that have not yet begun to invade the wall of the colon or rectum; these preinvasive lesions are not included in the cancer statistics.

Local: Cancers that have grown into the wall of the colon or rectum, but have not extended through the wall to invade nearby tissues.

Regional: Cancers that have spread through the wall of the colon or rectum and have invaded nearby tissue, or that have spread to nearby lymph nodes.

Distant: Cancers that have spread to other parts of the body, such as the liver or lung.

The stage of colorectal cancer depends on the extent of tumour spread through the layers of colon or rectum, nearby lymph nodes and distant organs and the most commonly used staging system is the TNM Staging system. Colorectal cancer is staged by combining cancer spreading information from three aspects: **T** stands for the growth of primary tumour into the wall of colon and nearby areas; **N** for the extent of tumour spreading to nearby (regional) lymph nodes; **M** for cancer metastasized to other organs of the body.

1.3 Clinically used drugs for colon cancer approved by (FDA) are:

1) Bevacizumab
2) Irinotecan HCl
3) Capecitabine
4) Cetuximab
5) Ramucirumab
6) Oxaliplatin
7) 5-Fluorouracil
8) Leucovorin
9) Calcium
10) Trifluridine and Tipiracil HCl
11) Panitumumab
12) Rogorafenib
13) Ziv-aflibercept

1.4 Drug Combination used for colon cancer approved by (FDA) are:

1) Capecitabine + Oxaliplatin
2) Leucovorin Calcium+5-Fluorouracil+Irinotecan
3) Leucovorin Calcium+5-Fluorouracil+Irinotecan+Cetuximab
4) Leucovorin Calcium+5-Fluorouracil+Oxaliplatin
5) 5-Fluorouracil+Leucovorin Calcium
6) Irinotecan+Capecitabine
7) Capecitabine+Oxaliplatin

1.5 Clinical Relevant Polymer Drug Conjugates

A number of polymer drug conjugates have been synthesized by medicinal chemists because of the routinely used chemotherapeutic agents in case of common tumours such as breast, prostrate, lung and colon. The chemotherapeutic agents are neither site specific nor free from adverse side effects. Therefore, with the aim of decreasing the side effects, increasing therapeutic index, much scientific research is in progress in the area of polymer drug conjugates **(Vicent and Duncan, 2006)**.

2. LITERATURE REVIEW

Numerous approaches which are useful for the delivery of drugs to the colon are discussed under the following heads:

2.1 Prodrug Approach

A prodrug is pharmacological inactive substance which upon biotransformation liberates biologically active parent drug molecule. In the prodrug approach, drug molecule is chemically modified through covalent bond, therefore, its chemical properties have been modified and it gets protected from the upper GIT. A large number of conjugates are available which have been designed and synthesized in the above mentioned approach such as Azo bond conjugates, Dextran conjugates, Cyclodextran conjugates, Glucuronate conjugates, Glycoside conjugates, Polypeptide conjugates and Polymeric prodrug **(Sinha and Kumaria, 2001)**.

2.2 Prodrug Conjugates

Different types of conjugates were used to prepare 5-Amino Salicylic Acid prodrugs, which are succeeded in releasing the 5-ASA in colonic region. They are biodegradable poly (ether-ester) azo polymers **(Samyn *et al.*, 1995)**, azo-linked polymeric prodrugs **(Schacht *et al.*, 1996)**, acrylic type polymeric prodrugs **(Davaran *et al.*, 1999)** and cyclodextrin prodrugs **(Uekama *et al.*, 1997).** Glucuronide prodrugs were developed for corticosteriods to deliver the drug to the large intestine of colitic rats **(Nolen *et al.*, 1997)**.

2.3 Azo bond Conjugates

The intestinal microflora plays an important role in a wide range of metabolic activities such as reduction of the nitro and azo groups in environmental and therapeutic compounds **(Rafii *et al.*, 1990, 1991; Walker and Ryan, 1971)**. Sulfasalazine i.e salicylazosulphapyridine (SASP) has been used for treatment of rheumatoid arthritis and anti-inflammatory disease **(Svartz *et al.*, 1970)**. A urethane-based analogue containing an azo aromatic linkage in the backbone was synthesized by reacting toluene -2, 6- diisocyanate with a mixture of an aromatic azo diol **(Chavan *et al.*, 2001)**.

2.4 pH-Dependent System

The pH plays an important role in case of colon targeted drug delivery. In the stomach, pH lies between 1-2 during fasting but increase after the intake of food. The different parts of colon have different pH values. The pH of transverse colon is 6.6, descending colon has 7.0, whereas cecum has pH 6.4. The selection of polymers for colonic delivery is also based upon pH range suitable for colon. It is also affected by diet and disease conditions. In ulcerative colitis pH values lies between 2.3 and 4.8 which have been measured in the proximal parts of the colon. pH dependent polymers has been used for the targeted delivery which should not dissolve at lower pH values i.e. stomach and small intestine and protect the drug formulation from the upper gastrointestinal tract but able to start disintegrate at neutral or slightly alkaline medium i.e. 6-7. Eudragit L has been used for colon targeting in patients with ulcerative colitis or Crohns disease **(Rajguru *et al*., 2011)**.

2.5 Time-Dependent System

The time dependent release system is based upon the principle of drug release until it reaches the colon so that targeted drug delivery is achieved. The main objective of this system is to protect the formulation from the acidic environment of the stomach and allowing further predefined lag time **(Shweta *et al*., 2006)**. The normal lag time for colon targeted drug delivery is 5 hours. The lag time of tablet was based on the thickness of the coating material. Hydroxy Propyl Methyl Cellulose (HPMC) compression coated tablets of 5-fluorouracil were studied for colon drug delivery based on time-dependent release of the drug. The release lag time and release rate of tablet was modified through adjusting the formulation variables such as weight and thickness **(Wu *et al*., 2007)**.

2.6 Glycoside Conjugates

This approach involves the use of prominent bacterial enzymes such as *glycosidases* e.g *β-D-galactosidase*, *β-D-glucosidase*, *α-L-arabinofuranosidase* and *β- D-xylopyranosidase* which are present in human feces for targeting the drug to the colon as stated by (**Friend, 1992)**. The enzymes present in the colon cleave the drug glycosides, which results the release of the free drug to be absorbed by mucosa of the colon. A number of studies have indicated that corticosteroids released from glycoside prodrugs are absorbed from the lumen of the large intestine and lead to much higher colonic tissue levels than are possible when the parent corticosteroid is administered systemically e.g. prodrugs of prednisolone, dexamethasone, hydrocortisone and fludrocortisone with D-galactosides and D-glucosides have been prepared **(Friend and Chang., 1984).**

2.7 Dextran Conjugates

Dextrans consist of repeated units of glucose moieties having hydroxyl groups which are used to link the drug with the polymer. The drugs having carboxyl groups are used for this approach and if there is absence of carboxyl group, succinic acid and glutaric acid are used as spacer between polymer and drug moiety **(Sinha and Kumria., 2003)**. This approach is also based upon the action of enzyme *dextranases.* The 5-ASA dextran conjugates were prepared and evaluated for the targeted delivery to colon **(Ahmed *et al*., 2006)**. The naproxen dextran conjugates were prepared and evaluated; the results showed that there was extensive release of drug naproxen in the cecum, homogenates of colon and small intestine **(Harboe *et al*., 1989).**

2.8 Cyclodextrin Conjugates

Cyclodextrins (CD's) are cyclic oligosaccharides having 6-8 glucose units joined together by α-1, 4-glucosidic linkage. It is well known that they are capable of being hydrolyzed and less absorbed through the stomach and small intestine and finally fermented by enzymes of colonic microfora into small saccharides. 5-Fluorouracil-1-acetic acid (5-FUA) was prepared and covalently conjugated to β-cyclodextrin (β-CyD) through ester or amide linkage, and the drug release behavior of the conjugates in enzymatic solutions and rat cecal contents were investigated **(Udo *et al*., 2010).**

2.9 Inulin Conjugates

Inulin has been conjugated to CoB_{12} vitamin, noradrenaline, and cysteine and has shown the conjugate can increase drug stability to light, temperature, hydrolysis, and chemical agents **(Schoener *et al*., 2013)**. The synthesis and characterisation of new hydrogel systems have also been designed for colon targeting. The gels were composed of methacrylated inulin, copolymerized with the aromatic azo agent bis (methacryloyl amino) azobenzene and 2-hydroxyethyl methacrylate or methacrylic acid. It has been shown that the uptake of water in the gels was inversely proportional to the methacrylic concentration, (methacryloyl amino) azobenzene and degree of substitution of the inulin backbone. The release of the drug prednisolone was studied in phosphate buffer, released 50% of drug after 5 hours **(Maris *et al*., 2001).**

2.10 POLYPHOSPHAZENE

Polyphosphazene is a biodegradable polymer having an inorganic backbone composed of alternating nitrogen and phosphorous atoms linked by alternating single and double bonds, with two substituents at each phosphorous atom. Polyphosphazene is the most versatile type of polymer because a tremendous variety of substituents can be attached to the polymeric backbone having phosphorous atom thus resulting in a very broad spectrum of chemical and physical properties which make them suitable for many applications such as vibration shock mounts, elastomers, biostructural materials, solid phase electrolytes and polymeric drug delivery systems **(Lakshmi *et al.*, 2003; Neilson and Wisian-Neilson, 1988)**. Polyphosphazenes have been used as polymeric carriers for biologically active agents. The main advantages of this polymer is mainly due to various properties that can be designed and prepared by nucleophilic substitution of polydichlorophosphazene with various pendent groups such as hydrophobic groups that confer water insolubility and protect the polymeric backbone against hydrolysis through groups that generate water solubility together with hydrolytic stability to side groups **(Song and Sohn, 1998).**

2.11 Synthesis of Polyphosphazenes

From the last two centuries, the polymerization process of hexachlorocyclotriphosphazene to polydichlorophosphazene has attracted considerable attention. The first attempt was done by Stokes in 1897 to polymerize the compound by heating at high temperature for a long time to produce an insoluble, intractable & useless elastomer known as inorganic rubber **(Stokes, 1897)** and Allcock performed the same reaction under strictly controlled conditions leading to soluble useful $(NPCl_2)n$ appropriate for further derivatization reaction which is still widely used in a large number of industrial and academic laboratories **(Allcock *et al.*, 1987)** and **(Kugel *et al.*, 1966).**

2.12 Reactivity of Poly (dichlorophosphazene)

The high reactivity of the polar P-Cl bond makes polyphosphazene $(NPCl_2)_n$ amenable to chloride replacement, without P=N bond cleavage, by a wide variety of inert organic or organometallic residues such as phenols or aliphatic alcohols, primary aromatic amines or primary and secondary aliphatic amines to form different classes of very interesting stable polyorganophosphazenes **(Lakshmi *et al.*, 2003).**

3. AIM AND OBJECTIVE

The aim of the research project is to synthesize and evaluate azo based polymeric prodrugs for colon cancer. In this approach, the anticancer drugs will be linked with biodegradable polymers such as polyphosphazene and inulin through azo linkage to have targeted delivery to the colon. The enzyme *azo reductase* present in the colon will act upon the polymer-linked prodrug and release the anticancer drug at the colon site only. Inulin has been conjugated to CoB_{12} vitamin, noradrenaline, and cysteine and the synthesized conjugates of inulin have been proved to increase drug stability to light, temperature, hydrolysis, and chemical agents. Besides conjugates, inulin has been extensively utilized as a matrix component, for drug delivery. This approach will not only deliver the anticancer drug at the colon but also improve therapeutic index and expected to be devoid of toxicity. The proposed technical approach for the synthesis of novel polymeric prodrugs is shown in Figure 2.

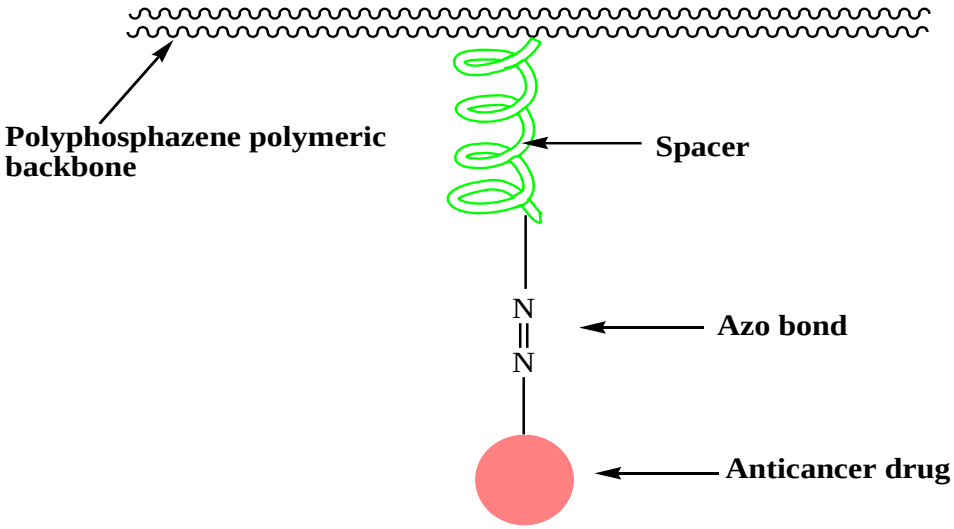

Figure 2. The proposed technical approach for the synthesis of polymer-linked prodrugs

Therefore, it is proposed to synthesize the prodrugs of polyphosphazene and inulin employing clinically used anticancer drugs such as 5-Fluorouracil, Erlotinib and Capecitabine etc. to have the desired targeted delivery of the drug to the cancerous colon.

4. RATIONALE

Anticancer drugs are neither site specific nor free from adverse toxic effects. Therefore, it was considered of interest to design and synthesize polyphosphazene and inulin linked prodrugs for colorectal cancer to have the targeted delivery of anticancer drugs to the colon site only.

In this approach clinically used anticancer drugs would be suitably linked to the drug carrier. Therefore, absorption and distribution of drug will depend on the physicochemical properties of the drug carrier and polymeric backbone. The *azo reductase* enzyme, present in the colon will act upon azo bond present in the polymer-linked prodrugs and release the anticancer drugs at the colon site only.

This approach will not only deliver the anticancer drugs at the target site but also have improved therapeutic index by minimizing the unwanted toxic side effects. This approach will also improve therapeutic index by minimizing interaction with non-targeting tissue, lower toxicity, reduced premature metabolism and excretion.

The proposed research project is novel in many aspects, It is proposed to synthesize and characterize polymer-linked prodrugs for the targeted delivery of the anticancer drugs to the colon site. The polymer-linked anticancer prodrug is expected to have the following advantages:

1. Protect the drug from absorption and metabolism until it reaches the site of action i.e. colon.
2. Localize the drug at the site of action.
3. Release the drug at cancerous colon site.
4. Minimize toxicity.
5. Chemical and biochemical stability.

Biodegradable polyphosphazenes, to be used as polymeric backbone, will be synthesized from the cheaper starting materials, ammonium chloride and phosphorous pentachloride.

The research work will be very useful with respect to target colon cancer on account of its site specificity and secondly it will be free from its undesired toxic side effects.

5. PLAN OF WORK AND METHODOLOGY

The research work to be carried out is discussed under the following headings:

(I) Synthesis of prodrugs of clinically used anticancer drugs such as 5-Fluorouracil, Erlotinib and Capecitabine etc.

(II) Synthesis of Hexachlorocyclotriphosphazene.

(III) Polymerization of Hexachlorocyclotriphosphazene to Poly(dichlorophosphazene).

(IV) Linking of prodrugs with Poly(dichlorophosphazene).

(V) Linking of prodrugs with Inulin.

(VI) *In-vitro* drug release studies of synthesized polymer-linked prodrugs.

(VII) *In-vitro* cell line studies

The novel polymer linked prodrugs to be synthesized in proposed research project entitled, "Synthesis and Evaluation of Novel Polymeric Prodrugs for the treatment of Colon Cancer" will be proved to deliver the drug at targeted site i.e Colon. The azo bond present in prodrugs will be specifically cleaved by *azo reductase* enzyme present in colon and augment the release of drug. Therefore, the proposed project will not only deliver the drug at specific site but also overcome side effects resulting from drug binding to non specific target tissue.

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